PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
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ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 August 1999 (27.08.99)	
International application No. PCT/EP98/08162	**Applicant's or agent's file reference** 6055 PCT
International filing date (day/month/year) 10 December 1998 (10.12.98)	**Priority date** (day/month/year) 12 December 1997 (12.12.97)
Applicant STUIVER, Maarten, Hendrik et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 July 1999 (02.07.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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Authorized officer

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Mogen International N.V.
Einsteinweg 97
2333 CB LEIDEN
Nederland

name and address of the party to whom the viability statement is issued

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Mogen International N.V. Address: Einsteinweg 97 2333 CB LEIDEN Nederland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: CBS 414.93 Date of the deposit or of the transfer: [1] Thursday, 12 August 1993

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on Friday, 13 August 1993 [2]. On that date, the said microorganism was

[X] [3] viable

[ ] [3] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY HAS BEEN PERFORMED [4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Centraalbureau voor Schimmelcultures Address: Oosterstraat 1 P.O. Box 273 3740 AG BAARN The Netherlands	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): drs F.M. van Asma Date: Friday, 13 August 1993

[4] Fill in if the information has been requested and if the results of the test were negative.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
6055 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 08162	10/12/1998	12/12/1997
Applicant		
MOGEN INTERNATIONAL NV et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

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   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   CONSTITUTIVE PLANT PROMOTERS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/08162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 106 739 A (COMAI LUCA ET AL) 21 April 1992 see the whole document ---	1,2
X	WO 92 18625 A (MOGEN INT) 29 October 1992 * see esp. p.10 l.19-37 * ---	1,2
X	EP 0 559 603 A (SANDOZ AG ;SANDOZ LTD (CH); SANDOZ AG (DE)) 8 September 1993 * see esp. p.3 l.49-57 * ---	1,2
X	WO 95 14098 A (CUI DECAI ;BIOTECHNOLOGY RES & DEV (US); NI MIN (US); GELVIN STANT) 26 May 1995 * see esp. p.11 l.8 - p.16 l.4, p.22 l.12 - p.23 l.5 * --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
30 March 1999	08/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
L	KONONOWICZ H. ET AL.: "Subdomains of the octopine synthase upstream activating element direct cell-specific expression in transgenic tobacco plants" THE PLANT CELL, vol. 4, no. 1, January 1992, pages 17-27, XP002065097 * this document was cited to confirm the expression pattern conferred by the ocs UAS disclosed in the prior art document WO95/14098 *	1-3
A	US 5 097 025 A (BENFEY PHILIP N ET AL) 17 March 1992 see the whole document	1-10
A	WO 94 12015 A (CHUA NAM HAI) 9 June 1994 cited in the application * see esp. p.6 l.9-27, p.23 l.1-5 *	1-10
A	WO 97 20056 A (CAMBRIDGE ADVANCED TECH ;GRAY JOHN CLINTON (GB); SANDHU JAGDEEP SI) 5 June 1997 see the whole document	9-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/08162

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5106739 A	21-04-1992	CA 1334175 A	31-01-1995
WO 9218625 A	29-10-1992	AU 663871 B	26-10-1995
		AU 1698992 A	17-11-1992
		BR 9205894 A	27-09-1994
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		SK 392392 A	09-08-1995
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		CA 2236166 A	05-06-1997
		EP 0870043 A	14-10-1998

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/82

A1

(11) International Publication Number: **WO 99/31258**

(43) International Publication Date: 24 June 1999 (24.06.99)

(21) International Application Number: PCT/EP98/08162

(22) International Filing Date: 10 December 1998 (10.12.98)

(30) Priority Data:
97203912.7 12 December 1997 (12.12.97) EP

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(75) Inventors/Applicants *(for US only)*: STUIVER, Maarten, Hendrik [NL/NL]; Groenhoevelaan 71, NL–2343 BR Oegstgeest (NL). SIJBOLTS, Floor, Hendrik [NL/NL]; Pastelstraat 144, NL–1339 JC Almere (NL).

(74) Agent: VAN WEZENBEEK, L., A., C., M.; Mogen International N.V., P.O. Box 628, NL–2300 AP Leiden (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.

(54) Title: CONSTITUTIVE PLANT PROMOTERS

(57) Abstract

The invention describes new promoters built from elements from a set of promoters which have a complementary expression pattern.

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CONSTITUTIVE PLANT PROMOTERS

FIELD OF THE INVENTION

The invention is directed to new plant promoters, more specifically those promoters which can be produced by assembling parts of promoters which have a complementary specificity.

BACKGROUND ART

Genetic engineering of plants has become possible by virtue of two discoveries: first of all the possibility of transformation of heterologous genetic material to the plant cell (most efficiently done by the bacterium *Agrobacterium tumefaciens* or related strains) and secondly by the existence of plant promoters which are able to drive the expression of said heterologous genetic material.

A typical plant promoter consists of specific elements. A basis is formed by the minimal promoter element, which enables transcription initiation, often accompanied by a sequence, also denominated as the TATA-box, which serves as a binding place for transcription initiation factors. In most promoters, the presence of this TATA-box is important for proper transcription initiation. It is typically located 35 to 25 basepairs (bp) upstream of the transcription initiation site. Another part of the promoter consists of elements which are able to interact with DNA-binding proteins. Known are G-box binding elements which are based on the hexanucleotide CACGTG motif. These elements have been shown to be able to interact with bZIP DNA-binding proteins which bind as dimers (Johnson & McKnight, Ann. Rev. Biochem, 58, 799-839, 1989). Other G-box related motifs, such as the Iwt and PA motifs have been described (WO 94/12015).

These motifs have been shown to be involved in tissue-specific promoter expression in plants. For instance, presence of Iwt tetramers confer embryo-specific expression, while PA tetramers confer high level root expression, low-level leaf expression and no seed expression.

Similarly, GT-1 like binding sites (grouped on basis of a moderate consensus sequence GGT$^{A}/_{T}$A) are described. Such a binding site is found far upstream the promoter region of the *Arabidopsis* plastocyanin promoter and seems to be involved in activation of transcription

during light periods (Fisschер, U. et al., Plant Mol. Biol. 26, 873-886, 1994).

Another sequence-related phenomenon which is found often in plant promoters is the presence of sequences which enable the formation of Z-DNA. Z-DNA is DNA folded in a left-handed helix which is caused by repeats of dinucleotides GC or AC. It is believed that folding in a Z-form influences the availability of the DNA for approach by RNA polymerase molecules, thus inhibiting the transcription rate.

One of the earliest and most important inventions in the field of plant protein expression is the use of (plant) viral and *Agrobacterium*-derived promoters that provide a powerful and constitutive expression of heterologous genes in transgenic plants. Several of these promoters have been used very intensively in plant genetic research and still are the promoter of choice for rapid, simple and low-risk expression studies. The most famous are the 35S and 19S promoter from Cauliflower Mosaic Virus (CaMV), which was already found to be practically useful in 1984 (EP 0 131 623), the promoters which can be found in the *Agrobacterium* T-DNA, like the nopaline synthase (nos), mannopine synthase (mas) and octopine synthase (ocs) promoters (EP 0 122 791, EP 0 126 546, EP 0 145 338). A plant-derived promoter with similar characteristics is the ubiquitin promoter (EP 0 342 926).

In time, several attempts have been made to increase the level of expression of these promoters. Examples for this are the double enhanced 35S promoter (US 5,164,316) and, more recently, the superpromoter, which couples parts of the *Agrobacterium* promoters (EP 729 514).

However, in many cases these promoters do not fulfill the criteria of an ideal promoter. All promoters described above show a clear pattern of organ- or developmental-specific expression, and frequently the pattern of expression found with these promoters is not ideal for some applications. Especially for biotechnological applications like the engineering of fungal and insect resistance, which require expression both in the right location as well as in the right timeframe of plant development there is a need for new

constitutive promoters which are able to give a high level of transgene expression at exactly the right time and place.

SUMMARY OF THE INVENTION

The invention provides for novel plant promoters, characterized in that they comprise 1) a minimal promoter and 2) transcription-activating elements from a set of promoters, which elements direct a complementary pattern and level of transcription in a plant.

More specifically, this plant promoter is a constitutive promoter in which each of the transcription-activating elements do not exhibit an absolute tissue-specificity, but mediate transcriptional activation in most plant parts at a level of ≥1% of the level reached in the part of the plant in which transcription is most active. An example of such promoter pairs is a set of promoters in which one is most active in green parts of the plant, while the other promoter is most active in underground parts of the plant. More specifically the new promoter is a combination of the ferredoxin and the RolD promoter. Preferably in this construct the minimal promoter element is derived from the ferredoxin promoter and the ferredoxin promoter is derived from *Arabidopsis thaliana*. The rolD promoter is derived from *Agrobacterium rhizogenes*.

Also part of the invention is a plant promoter which is a combination of the plastocyanin and the S-adenosyl-methionine-1 promoter, whereby preferably the minimal promoter element is derived from the S-adenosyl-methionine-1 promoter and both the plastocyanin promoter and the S-adenosyl-methionine-1 promoter are derived from *Arabidopsis thaliana*.

Further part of the invention are chimaeric gene constructs for the expression of genes in plants comprising the above disclosed promoters.

DESCRIPTION OF THE FIGURES

Figure 1: Schematic representation of pMOG410 and pMOG1059

Figure 2: Distribution of GUS expression of potato lines transformed with the constructs pMOG1059 en pMOG410. GUS staining was judged visually and classes of expression, relative to the highest GUS expression measured in our lab (set at 4). A value of zero indicates no visible expression.

Figure 3: Graphic representation of the average expression of GUS enzyme in primary transformants of tomato, oilseed rape and potato. GUS expression was determined visually and compared to a high level expressing 35S GUS transgenic tobacco plant ranking a score of 4. Standard deviation of the measured values are indicated on each of the bars.

Figure 4: Graphic representation of the distribution of potato plants with various levels of GUS expression containing SAM-1-, Pc-35S- and PcSAM1-GUS constructs. Scored are expression in leaf mesophyll, leaf vascular system, stem and root.

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of this specification the following definitions are valid:

A **promoter** consists of an RNA polymerase binding site on the DNA, forming a functional transcription initiation start site. A promoter usually consists of at least a TATA box and possibly of other sequences surrounding the transcription initiation site (initiator) and can either be used isolated (minimal promoter) or linked to binding sites of transcription-activating elements, silencers or enhancers that may enhance or reduce transcription initiation rates, and which may function respective of developmental stage, or external or internal stimuli.

The initiation site is the position surrounding the first nucleotide which is part of the transcribed sequence, which is also defined as position +1. With respect to this site all other sequences of the gene and its controlling regions are numbered. Downstream sequences (i.e. further protein encoding sequences in the 3' direction) are denominated positive, while upstream sequences (mostly of the controlling regions in the 5' direction) are denominated negative.

A **minimal promoter** is a promoter consisting only of all basal elements needed for transcription initiation, such as a TATA-box and/or initiator.

An **enhancer** is a DNA-element which, when present in the neighbourhood of a promoter is able to increase the transcription initiation rate.

A promoter is **constitutive** when it is able to express the gene that it controls in all or nearly all of the plant tissues during all or nearly all developmental stages of the plant.

Specific expression is the expression of gene products which is limited to one or a few plant tissues (spatial limitation) and/or to one or a few plant developmental stages (temporal limitation). It is acknowledged that hardly a true specificity exists: promoters seem to be preferably switch on in some tissues, while in other tissues there can be no or only little activity. This phenomenon is known as **leaky expression**. However, with specific expression in this invention is meant preferable expression in one or a few plant tissues.

The **expression pattern** of a promoter (with or without enhancer) is the pattern of expression levels which shows where in the plant and in what developmental stage transcription is initiated by said promoter.

Expression patterns of a set of promoters are said to be complementary when the expression pattern of one promoter shows little overlap with the expression pattern of the other promoter.

The level of expression of a promoter can be determined by measuring the 'steady state' concentration of a standard transcribed reporter mRNA. This measurement is indirect since the concentration of the reporter mRNA is dependent not only on its synthesis rate, but also on the rate with which the mRNA is degraded. Therefore the steady state level is the product of synthesis rates and degradation rates. The rate of degradation can however be considered to proceed at a fixed rate when the transcribed sequences are identical, and thus this value can serve as a measure of synthesis rates. When promoters are compared in this way techniques available to those skilled in the art are hybridisation S1-RNAse analysis, Northern blots and competitive RT-PCR. This list of techniques in no way represents all available techniques, but rather describes commonly used procedures used to analyse transcription activity and expression levels of mRNA.

One of the technical difficulties encountered in such an analysis is that the qualitatively best results can only be obtained by fusing transcriptional activating parts to the reporter RNA molecule, in such

a way that only reporter sequences are transcribed. This requires the exact determination of the RNA synthesis start, and joining at that point the sequences of the reporter mRNA.

This is important for a number of reasons. First, the analysis of transcripion start points in practically all promoters has revealed that there is usually no single base at which transcription starts, but rather a more or less clustered set of initiation sites, each of which accounts for some start points of the mRNA. Since this distribution varies from promoter to promoter the sequences of the reporter mRNA in each of the populations would differ from each other. Since each mRNA species is more or less prone to degradation, no single degradation rate can be expected for different reporter mRNAs. Secondly, it has been shown for various eukaryotic promoter sequences that the sequence surrounding the initiation site ('initiator') plays an important role in determining the level of RNA expression directed by that specific promoter. This includes also part of the transcribed sequences. The direct fusion of promoter to reporter sequences would therefore lead to much suboptimal levels of transcription.

Leaving in these transcribed sequences does allow determining the transcription rates, but potentially alters the stability of the reporter mRNA and influences translation initiation rates of an eventual open reading frame.

The role of this analysis, however, is the determination of the relative level of constitutive expression of a heterologous protein, as is the most frequent used application in biotechnology. Therefore the most important parameter is the ability of the tested sequences to drive high level expression of a heterologous reporter protein.

This would involve coupling the coding sequences of a reporter protein to the transcription activating part, promoter and 5' untranslated sequence of the gene which is tested for its properties. In this way a complex set of effects (combining transcription rates, mRNA stability (and thus degradation rates of the mRNA) and translational initiation rates) is reduced to one value that is a very useful value for determining usefulness of the tested gene elements in biotechnological applications.

There is no current word or phrase to describe this value. In the course of this application next to the term '**expression value**' the terms 'expression level' and 'transcriptional activity' are used. We realize that this may cause some confusion. In all cases we do indicate with these and related terms the value just mentioned.

A commonly used procedure to analyse expression patterns and levels is then through determination of the 'steady state' level of protein accumulation in a cell. Commonly used candidates for the reporter gene, known to those skilled in the art are ß-glucuronidase (GUS), Chloramphenicol Acetyl Transferase (CAT) and proteins with fluorescent properties, such as Green Fluorescent Protein (GFP) from *Aequora victoria*. In principle, however, many more proteins are suitable for this purpose, provided the protein does not interfere with essential plant functions. For quantification and determination of localization a number of tools are suited. Detection systems can readily be created or are available which are based on e.g. immunochemical, enzymatic, fluorescent detection and quantification. Protein levels can be determined in plant tissue extracts or in intact tissue using in situ analysis of protein expression.

Generally, individual transformed lines with one chimeric promoter-reporter construct will vary in their levels of expression of the reporter gene. Also frequently observed is the phenomenon that such transformants do not express any detectable product (RNA or protein). The variability in expression is commonly ascribed to 'position effects' although the molecular mechanisms underlying this inactivity are usually not clear.

The term **average expression** is used here as the average level of expression found in all lines that do express detectable amounts of reporter gene, so leaving out of the analysis plants that do not express any detectable reporter mRNA or -protein.

Root expression level indicates the expression level found in protein extracts of complete plant roots. Likewise, 'leaf' and 'stem expression levels' are determined using whole extracts from leaves and stems. It is acknowledged however, that within each of the plant parts just described, cells with variable functions may exist, in which promoter activity may vary.

For the promoters described in this application the expression levels in large plant parts, containing cells with various functions, are measured. However, more detailed analyses may contribute to

construction of a promoter that is even 'more constitutive' taking into account that more celltypes within a plant part are taken into account.

As a standard for judging expression levels the 35S promoter of the Cauliflower Mosaic Virus is a convenient and widespread used standard. The average expression level of this promoter may be classified as medium high.

The invention shows that it is possible to combine elements from one promoter which are responsible for a specific expression with elements from another promoter which are responsible for a complementory expression pattern to form a promoter which - as a result - shows expression in the tissues and developmental stages which form part of the expression pattern of both promoters. If the complementation results in activity in (nearly) all the cells of the plant, such complementation will yield a constitutive promoter. It seems to be necessary, however, that both promoters have a low expression value in the tissues and developmental stages which are specific for the other promoter. It has been established that, for being suitable, the transcriptional activity in the plant parts where expression is low should be preferably ≥1% of the level of transcription which is reached in the plant parts where transcriptional activity is high.

This limits the availability of promoters and promoter elements from which to build a new constitutive promoter. A suitable promoter-pairs which fulfills the above mentioned criteria is:

- the ferredoxin promoter in combination with the rolD promoter
- the S-adenosyl methionine promoter in combination with the plastocyanin promoter

Other promoter-pairs which are complementary and which show at least some expression in the tissues and developmental stages which are specific for the other promoter can also be applied.

Delineation of promoter and/or enhancer parts needed.

Whereas transcription-regulating elements, especially in eukaryotes, may be present at large distances from the promoter/transcription initiation site, and located both downstream or upstream of the initiation site, many plant genes have most of their regulatory elements in the area directly upstream of the promoter. In order to

identify the main transcription-activating elements of promoters it is common procedure to link parts of the non-transcribed areas that are found upstream (and downstream) of the promoter to a reporter gene, to analyse the ability of each of the truncated DNA elements to direct expression of that reporter. For delineation of more promoter-proximal sequences involved in transcription regulation, fragments of the enhancer sequences are most commonly coupled to a promoter, which may be derived from the gene of which transcription regulation is studied. Alternatively, a heterologous promoter can be used such as the sequences of the 35S promoter from -46 to +4, relative to the transcription start, which is functionally coupled to a reporter gene as described above.

In this way it is possible to delineate the transcription activating elements of most genes, a process that is well-known to those skilled in the art.

A large number of transcription regulatory elements of genes have been analysed in such a manner, and data relevant for this analysis are directly available to those skilled in the art through scientific publications.

Transcription activating elements that on average can direct expression to approximately the average level of the 35S promoter (at least 50% of this level) in at least some of the plant parts, and that are also capable of directing at least 0.5% (of the 35S level) transcription in other plant parts are then selected for further use.

The minimal promoter element is typically derived from one of the promoters of the promoter-pair, although not necessarily. It can be envisaged that such a minimal element is derived from a third promoter or is even made synthetically.

Based on the results of the analysis described above, transcription activating parts with complementary activities are selected. That is, for example, a promoter with expression throughout the plant, transcription activating DNA fragments that direct high level root expression and with lower leaf and stem expression levels, are combined with elements that direct expression mainly in the leaf and stem, but lower in the root. Other combinations of complementary transcription activating parts are obvious.

Preferentially, the level of expression in the parts where expression is lowest does not fall below 1% of the level obtained in the highest

part. More preferred is the situation where the relation between lowest expression and highest expression between plant parts is larger than 5%.

This coupling can most easily be done by known genetic engineering techniques. The gene which has to be expressed by the new constutive promoter can be cloned behind the promoter. It is adviseable to build in a unique NcoI-cloning site at the linkage of the 5' untranslated sequence attached to the promoter to allow precise junction of the open reading frame (ORF) and the 3' end of the promoter in which the gene of interest can be inserted.

The ferredoxin-rolD pair.

One of the preferred combinations of the present invention is a constitutive plant promoter comprising elements of both the ferredoxin promoter and the rolD-promoter. Preferably the ferredoxin promoter is obtained from *Arabidopsis thaliana* where it drives the ferredoxin A gene, a gene which is involved in the photosynthesis. The expression of this gene and the responsiveness of its promoter to light has been reported (Vorst, O. et al., Plant Mol. Biol. 14, 491-499, 1990; Vorst, O. et al., The Plant J. 3(6), 793-803, 1993; Dickey, L.F. et al., The Plant Cell 6, 1171-1176, 1994). Since the ferredoxin gene is involved in photosynthesis the promoter is most active in green tissue. mRNA levels were shown to be high in chloroplast-containing organs such as stem, leaves and bracts, but also in young growing tissues, such as whole flowers and seedlings. Interestingly, there is a smaller, but significant expression in soilborne areas of the plant. The promoter sequence contains both a G-box and an I-box containing region. Also a potential Z-folding DNA sequence is found at position -182.

The rolD promoter is reported to have strong expression in the roots and is obtainable from *Agrobacterium rhizogenes*. Although the source organism is a bacterium, the promoter is very suitable for expression in plants because the bacterium is a phytopathogen which causes hairy-root disease in plants. For that purpose it transfers DNA to the plant amongst which the rolD gene is responsible for root elongation. To be expressable in plants this gene needed a strong promoter functional in plants, the rolD promoter. GUS-studies have shown that expression under control of the rolD-promoter yields mainly root-specificity (Leach, F. and Aoyagi, K., Plant Sci. 79, 69-76, 1991). Also, some expression in leaves was observed.

A combination of the ferredoxin and the rolD promoter can be obtained in two ways, depending on from which promoter the minimal promoter element and 5' untranslated sequences will be taken. In our examples we have used the minimal promoter element from the ferredoxin promoter, but deriving it from the rolD promoter is equally well possible.

The S-adenosyl-methonine synthetase and plastocyanin pair.

Another favorable promoter can be obtained from a combination of the S-adenosyl-methionine synthetase (SAM) promoter and specific parts of the plastocyanin promoter. Preferably, both promoters are obtained from *Arabidopsis thaliana*.

The SAM promoter regulates the expression of S-adenosyl-methionine synthetase, which is an enzyme active in the synthesis of polyamines and ethylene. Promoter studies showed a strong expression in vascular tissues, in callus, sclerenchyma and some activity in root cortex (Peleman, J. et al., The Plant Cell 1, 81-93, 1989) which was reasoned to be due to the involvement of the enzyme in lignification.

The plastocyanin promoter, like the ferredoxin promoter, is also a promoter which is active in the photosynthetic pathway. mRNA levels are high in green, chloroplast-containing structures, such as leaves, cauline leaves, stem and whole seedling. Also in flowers the promoter is very active. Little expression is detectable in silique, seed and root (Vorst, O. et al., The Plant J. 4(6), 933-945, 1993).
By combining these specificities it is possible to create a chimeric promoter that drives good expression both in the photosynthetic areas of the leaf and stem, as well as in the area's not involved in photosynthesis, such as the cells forming and surrounding the vascular system in leaves and stems.

Other pairs of promoters.

The above given examples of promoter-pairs show in both cases the presence of a promoter which is active during photosynthesis. It is envisaged that other promoters which are regulating expression of a gene needed for photosynthetic activity may be suitable for a combination with either the rolD or other root-preferential promoters.

In the construction of a promoter that drives expression throughout the plant: if one of the components is a promoter which is more or less specific for green parts, this automatically means that

the other promoter of the pair should be predominantly (but not exclusively) expressed in the roots and other non-photosynthesizing organs.

In the construction of a promoter that drives expression in all parts of leaves and stems, the combination may be made by using a promoter which is more or less specific for green parts and a promoter which drives expression primarily in the vascular system.

However, the invention is not limited to the combination of a root-preferential and a green part-preferential promoter, and a combination of green-part-preferential and vascular system-preferential promoters. All promoter combinations provided that the expression patterns of the individual promoters are complementary can be used.

It is also possible that the elements from which e.g. a new constitutive promoter is composed are derived from a set with more than two promoters. The above discussed complementarity should then also exist.

EXPERIMENTAL PART

Example 1

Cloning of the chimeric Fd-rolD promoter:

A 512 bp *Arabidopsis thaliana* ferredoxin promoter fragment (O. Vorst et al., 1990, PMB 14, 491-499.) ranging from position -512 to +4 (relative to the ATG startcodon of the ferredoxin Open Reading Frame) was isolated by digestion with HincII and NcoI. This fragment contains most of the transcriptional regulatory sequences of the ferredoxin promoter, the promoter sequences and leader of the ferredoxin transcript. An XbaI site was introduced, for cloning reasons, at positions -5 to -10 relative to the ATG (O. Vorst et al., 1990, PMB 14, 491-499.). This changes the original sequence of the clone at this point from ACAAAA to TCTAGA (SEQ ID NO: 1).

Part of the *Agrobacterium rhizogenes* rolD upstream sequences (SEQ ID NO: 2) (Leach et al., 1991 Plant Sci. 79, 69-76) were fused to the ferredoxin promoter sequences described above. A HindIII-RsaI fragment, comprising nucleotides -385 to -86 relative to the initiation codon was cloned next to the ferredoxin fragment, joining the RsaI sites of the latter with the HincII site of the former. This chimaeric element, containing the promoter and some of the activating sequences of the ferredoxin gene, and upstream activating sequences of the rolD gene was used in subsequent studies as to its transcription-stimulating properties (SEQ ID NO: 3).

Example 2

GUS-fusions

The Fd-rolD chimaeric promoter/activator was coupled to the GUS gene, engineered to contain an intron gene (Jefferson *et al.*, (1987) EMBO J 6: 3901-3907). The NcoI restriction site on the ATG start codon was used to join the promoter to the Open Reading Frame (ORF) of the GUS gene, coupled to a 265 bp fragment containing the Proteinase Inhibitor II 3' untranslated and transcriptional termination sequences (Thornburg *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84, 744-748; An *et al.*, Plant Cell 1, 115-122).

The whole expression cassette, containing the promoter, GUS gene and 3' PI-II sequences was cloned out using BamHI and EcoRI and introduced into the binary vector pMOG800 (deposited at the Centraal Bureau voor

Schimmelcultures, Baarn, The Netherlands, under CBS 414.93, on august 12, 1993) digested with the same enzymes. The subsequently made construct (pMOG1059) was used in transformation experiments with various plants. As a control a 35S CaMV promoter-GUS construct was used. This is construct pMOG410. A schematic representation of both constructs is found in figure 1.

Example 3

Expression levels and patterns of promoter activity during early stages of plant transformation

First, *Arabidopsis thaliana* transformants were made with both constructs and GUS expression was followed in time during the transformation procedure.

GUS expression levels were determined visually, on a scale of 0 to 5, where 0 is no detectable expression and 5 is the highest level of GUS we have observed in leaves of a transgenic plant, of a rare tobacco 35S-GUS-transgenic (line 96306). Samples from leaves of this plant were included in all experiments for internal reference.

In table 1 the relative GUS expression in *Arabidopsis thaliana* explants is indicated, at several times after *Agrobacterium tumefaciens* cocultivation (DAC; days after cocultivation)

Table 1. relative GUS activity of Arabidopsis root explants.

Construct : Time of assay	pMOG1059	pMOG410
DAC 0	2	3
DAC 2	3	3
DAC 5	3	3
DAC 7	4	3
DAC 9	4	3
DAC 12	4	3

As can be seen from this comparison, GUS expression driven by the chimaeric promoter starts slightly later after cocultivation but from day 7 on, exceeds the level of expression obtained with the reference 35S promoter.

Very similar data were obtained when *Brassica napus* explants were scored for GUS expression. At day 5 after co-cultivation the 35S promoter is slightly higher, but the situation is reversed on day 20 after co-cultivation. Also for tomato similar data were obtained. Here

even at the earliest stage of analysis expression of pMOG1059-transgenics exceeded that of pMOG410 transgenics.

Example 4

Expression levels and patterns in *in vitro* grown plants

When plants are grown up further, differences between these promoters become ever clearer. Leaf samples of fully regenerated plants were analysed for GUS expression. Averages were obtained from 11-48 plants, dependent on the construct.

For *Arabidopsis thaliana* that was grown in vitro only, no large difference was observed between GUS expression in pMOG1059 and pMOG410-transgenics.

Table 2. Average relative GUS activity of leaf samples of all tested crops.

Construct : Crop :	pMOG1059	pMOG410
Potato	4.0	2.1
Brassica napus	3.7	2.8
Arabidopsis	4.0	4.1
Tomato	2.2	2.1

What is also clear from the data presented in figure 2 that a significant number of 35S-GUS transgenic lines (app. 50% was found repeatedly in our experiments) do not express GUS to a level that it is visible. So not only maximum and average expression are higher in the Fd-rolD-GUS transgenics, also the frequency with which transgenic plants do express GUS is strongly enhanced. In about 50 transgenic potato plants carrying the Fd-rolD-GUS construct, we have found no weak expressor, suggesting a reliable high expression in at least 98% of the lines made.

Example 5

Comparison of promoter performance in various crops

Constructs pMOG410 (35S-GUS) and pMOG1059 (Fd-rolD-GUS) were also introduced into oilseed rape and tomato for a further comparison of promoter performance. Also the data for potato are included here.

As shown in Figure 3A, in tomato the overall level of expression of the Fd-rolD promoter is higher both at the latest stage of *in vitro* growth as well as in leaves of 4 and 7 week old plants. Also in stems of 7 weeks this holds true, however, for roots, an average weaker expression is observed with the Fd-rolD promoter than for the 35S promoter.

Also in oilseed rape and potato, similar results are obtained, with the notable exception that in potato roots the level of expression by the Fd-rolD promoter exceeds that of the 35S promoter. As shown in figures 3B and 3C both the average expression of the Fd-rolD promoter is higher and also the variation in expression is significantly lower. In conclusion we can say we have created a promoter that withstands the comparison with the 35S promoter easily in three major crops.

Example 6

Expression of nptII transgene.

In order to also check usability of the Fd-rolD promoter for other purposes, the promoter was linked to the nptII gene, of which expression of the corresponding gene product confers resistance in plants to the antibiotic kanamycin. This element was placed between the left and right borders of the T-DNA allowing *Agrobacterium tumefaciens*-mediated transfer to plants. As a control, similar constructs in which the expression of the nptII gene was under control of the nos promoter were used.

The resistance to kanamycin in transgenic potato plants is manifested by the development of transgenic calli and shoots during a standard transformation procedure, in which kanamycin is used in the culture medium.

On average, for the constructs with the nos-nptII selction cassette, the transformation frequency for potato is 45%, for constructs with the Fd-rolD-nptII selection cassette the frequency is on average 61%. While we do not know at this moment how relevant the increase in transformation frequency is for this construct, it indicates that the Fd-rolD promoter is at least as suitable for driving a heterologous gene such as nptII, as commonly used constitutive promoters such as nos.

Example 7

Comparing visual scoring to quantitative values

From the analysis of GUS expression based on 1) histochemical analysis and scoring to an internal control and 2) to a quantitative analysis of GUS enzymatic activity, we have learned that both give a reproducible quantitative figure. A thorough analysis of both scores for tomato and oilseed rape leaves and roots, leads to the conclusion that scale 3, which compares best to that of 35S, equals about 2000 pmol MU/minute.mg made in the quantitative analysis. In scale 1 and 2, averages are 1000 and 1500, respectively, which set the value of 50% of 35S. About 1% expression of that level equals 100 pmol MU/minute.mg, which is frequently under the detection level for histochemical detection , although sometimes detectable as very light blue staining due to GUS expression. Therefore one can use histochemical staining as a marker for promoter efficacy, by measuring the level of blue staining, and use these data to select promoter elements of use.

Example 8

Construction of the SAM1 promoter and fusion to GUS.

For the construction of the SAM1 promoter genomic DNA (SEQ ID NO: 4) was isolated from *Arabidopsis thaliana* Landsberg erecta leaves using a CTAB extraction procedure. Primers were designed based on the published sequence of the SAM1 gene from *Arabidopsis thaliana* K85 (Peleman et al., (1989) Gene 84, 359-369). In a PCR (30 cycles of 45 seconds 95 °C, 45 seconds 50°C and 1' 72°C; same program was used in all other PCR's described in this part) the promoter element was amplified using primers FR-Psam-143 5' AGA TTT GTA TTG CAG CGA TTT CAT TTT AG 3' (SEQ ID NO: 5) and FR-Psam-216 5' ATC TGG TCA CAG AGC TTG TC 3' (SEQ ID NO: 6) yielding a fragment of about 550 bp. The DNA fragment was isolated from an agarose gel and cloned into the pGEM-T vector (Promega Corp., Madison WI, USA). This clone was used as a template to introduce a *Nco* I site at the translation start by PCR using primers FR-Psam-144 5' GTC TCC ATG GTG CTA CAA AGA ATA G 3' (SEQ ID NO: 7) and FR-Psam-143. The resulting 500 bp fragment was cloned in the pGEM-T vector. The *Eco*R I and *Hin*d III sites located in the promoter region were removed by PCR in two steps using this clone

as a template. In this PCR a *BamH* I site was introduced upstream of the SAM1 promoter and a *Hind* III site was introduced at the 3' site of the promoter. In the first PCR step three promoter fragments were generated. The first fragment (1) will contain the 5' *BamH* I site and the mutated *EcoR* I site using primers FR-Psam-248 5' CGG GAT CCT GCA GCG ATT TCA TTT TAG 3' (SEQ ID NO: 8) and FR-Psam-249 5' ACA TGA ACG AAT GCA AAA TCT C 3'(SEQ ID NO: 9). The middle fragment (2) is obtained with primers FR-Psam-250 5' AGA TTT TGC ATT CGT TCA TGT G 3' (SEQ ID NO: 10)and FR-Psam-251 5' TGT AAG CAT TTC TTA GAT TCT C 3'(SEQ ID NO: 11). This fragment has a partial overlap with fragment 1 and 3 and has mutated *EcoR* I and *Hind* III sites. The third PCR fragment (3) will contain the mutated internal *Hind* III site and introduces a *Hind* III site at the 3' end of the promoter encompassing the *Nco* I site at the translation start and is generated using primers FR-Psam-252 5' AAG AAA TGC TTA CAG GAT ATG G 3' (SEQ ID NO: 12) and FR-Psam-253 5' GAC AAG CTT GAT CCC ATG GTG CTA CAA AGA ATA G 3'(SEQ ID NO: 13). In a second PCR the 3 fragments 1,2 and 3 were mixed together in one tube and amplified with primers FR-Psam-248 and FR-Psam-253. Due to the overlap between fragments 1 and 2, and 2 and 3, this PCR yields the complete mutated promoter. After digestion with *BamH* I and *Hind* III the resulting SAM1 promoter was cloned in a pBSK+ vector. The SAM1 promoter was then cloned into a vector containing a GUSintron-TPI-II reporter cassette by exchanging the upstream region using the *BamH* I and *Nco* I restriction sites. This was done by digestion of the SAM1 clone with *BamH* I and *Nco* I and isolation of the promoter fragment from a agarose gel. The GUS vector was digested with the same enzymes and the vector was then isolated from a agarose gel thus discarding the original upstream sequences promoter.

The SAM1 promoter-GUSintron-TPI-II reporter cassette was then cut out of the vector by BamH I and EcoR I digestion after which the reporter cassette was isolated from a agarose gel and cloned into the binary vector pMOG800 digested with BamH I and EcoR I. The resulting binary vector pMOG1402 was introduced in *Agrobacterium tumefaciens* strain EHA105 for transformation to potato.

Example 9

Construction of the Pc-SAM1 chimaeric promoter and fusion to the GUS gene

The plastocyanin enhancer (Pc) from Arabidopsis thaliana Col-0 was obtained by PCR. Therefore primer FR-Pc-146 (5'agt ggt acc atc ata ata ctc atc ctc ctt ca3') (SEQ ID NO: 14) and primer FR-Pc-247 (5'cga agc ttt aca aat cta att tca tca cta aat cgg a3') (SEQ ID NO: 15) were developed introducing a *Kpn* I restriction site upstream of the enhancer and a *Hind* III restriction site downstream of the plastocyanin enhancer. The PCR was performed using Cloned *pfu* DNA polymerase (Stratagene) for 30 cycles 1' 95°C, 1' 50°C , 4' 72°C and 1 cycle 1' 95°C, 1' 50°C , 10' 72°C. The resulting PCR fragment was ligated into a high copy cloning vector using *Kpn* I and *Hind* III resulting in construct pPM15.1.

This clone was used as a template for a PCR (30 cycles of 1' 95 °C, 1' 50°C and 2' 72°C) using primers FR-Pc-145 5' GCT GCA ATA CAA ATC TAA TTT CAT CAC TAA ATC GG 3' (SEQ ID NO: 16) and FR-Pc-146 5' AGT GGT ACC ATC ATA ATA CTC ATC CTC CTT C 3'(SEQ ID NO: 14). The PCR generates a fragment of about 850 bp encompassing the Pc enhancer (SEQ ID NO: 17) containing a upstream *Kpn* I site and overlap with the 5' side of the SAM1 promoter (see Example 8). The PCR fragment was then mixed with a PCR fragment of the SAM1 promoter generated with primers FR-Psam-143 and FR-Psam-144 using the pBKS+ clone containg the adjusted SAM1 promoter described in example 8. In a PCR on this mixture the PcSAM chimeric promoter was generated using primers FR-Psam-144 and FR-Pc-146. The resulting promoter fragment of about 1.3 kb (SEQ ID NO: 18) was isolated from a agarose gel after digestion with *Kpn* I and *Nco* I and then cloned into a high copy cloning vector (pUC28) digested with the same enzymes. The promoter fragment was then cut out of this vector by digestion with BamHI and NcoI and cloned in front of the GUSintron gene as described above in Example 8. The complete Pc-SAM-GUS-TPI-II reporter cassette was then cloned into pMOG800 as described for the SAM1-GUS-TPI-II reporter cassette in Example 8. The resulting binary vector pMOG1400 was introduced in *Agrobacterium tumefaciens* strain EHA105 for transformation to potato.

Example 10

Construction of the Pc enhancer-35S promoter and fusion to the GUS gene

The plastocyanin enhancer (Pc) from Arabidopsis thaliana Col-0 was obtained by PCR (see above).
This clone was used as a template for a PCR (30 cycles of 1' 95 °C, 1' 50oC and 2' 72°C; all other PCR reactions described in this part were carried out with the same program) using primers FR-Pc-291 5' GTC TTG TAC AAA TCT AAT TTC ATC ACT AAA TCG G 3' (SEQ ID NO: 19) and FR-Pc-146 5' AGT GGT ACC ATC ATA ATA CTC ATC CTC CTT C 3' (SEQ ID NO: 14). The PCR generates a fragment of about 850 bp encompassing the Pc enhancer containing a upstream KpnI site and overlap with the 5' side of the minimal 35S promoter. The minimal 35S promoter was obtained in a PCR using pMOG971 as a template (containing the 35S promoter and omega 5' UTR) and primers FR-35S-292 5' TTA GAT TTG TAC AAG ACC CTT CCT CTA TAT AAG G 3'(SEQ ID NO: 20) and ls19 (SEQ ID NO: 21). The resulting fragment has overlap with the Pc enhancer and contains a internal NcoI site at the translation start. The two PCR fragments were then mixed and a PCR reaction was carried out using primers FR-Pc-146 and ls19. The resulting fragment was then digested with KpnI and NcoI, isolated from a agarose gel and cloned in pUC28 digested with the same enzymes. The resulting clone was, subsequently, digested with BamHI and NcoI, and the promoter fragment (SEQ ID NO: 22) was isolated from a agarose gel and cloned upstream of the GUS gene as described in Example 7. The complete reporter cassette was then introduced in the binary vector pMOG800 as described in Example 7. The resulting binary vector pMOG1401 was introduced in *Agrobacterium tumefaciens* strain EHA105 for transformation to potato.

Example 11

Expression levels and patterns in in vitro grown plants

Transformed plants were grown up, and leaf samples of fully regenerated plants were analyzed for GUS expression. In figure 4 the analysis of expression in leaf mesophyll, leaf vascular system, stems and roots is indicated. A very low level of GUS staining was observed in the mesophyll part of leaves of SAM1-transgenic plants although the scoring indicates a GUS expression level of 0.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Mogen International N.V.
Einsteinweg 97
2333 CB LEIDEN
Nederland

name and address of depositor

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
E. coli DH5 alpha strain / the plasmid pMOG800	CBS 414.93

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[X] a scientific description

[ ] a proposed taxonomic designation

(mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary accepts the microorganism identified under I above, which was received by it on Thursday, 12 August 1993 *(date of the original deposit)*[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on not applicable *(date of the original deposit)* and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on not applicable *(date of receipt of request for conversion)*

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Centraalbureau voor Schimmelcultures

Address: Oosterstraat 1
P.O. Box 273
3740 AG BAARN
The Netherlands

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

drs F.M. van Asma

Date: Friday, 13 August 1993

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page) CBS/9107


pMOG410
LB
nos-nptII
P1-II
GUS + intron
RB 35S CaMV
pMOG1059
LB
nos-nptII
P1-II
GUS + intron
RB Fd-rolD chimeric

Fig. 1

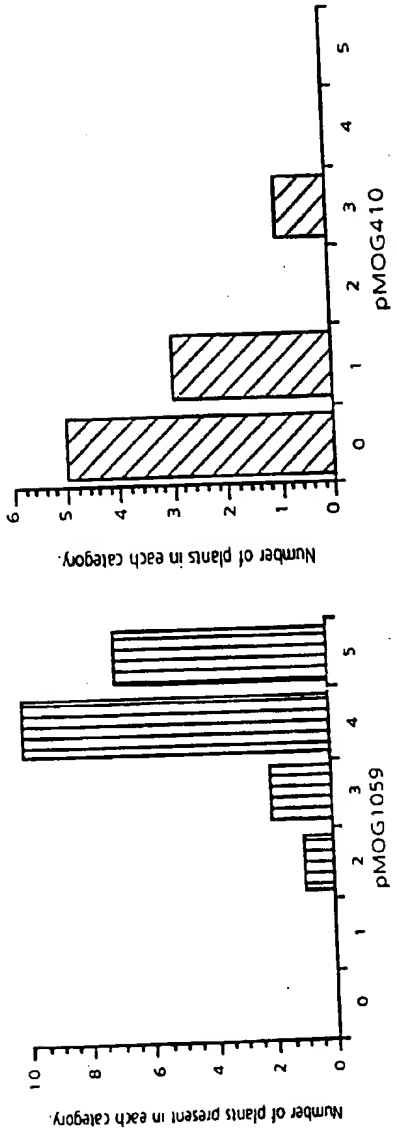
pMOG410
Number of plants in each category
pMOG1059
Number of plants present in each category

Fig. 2

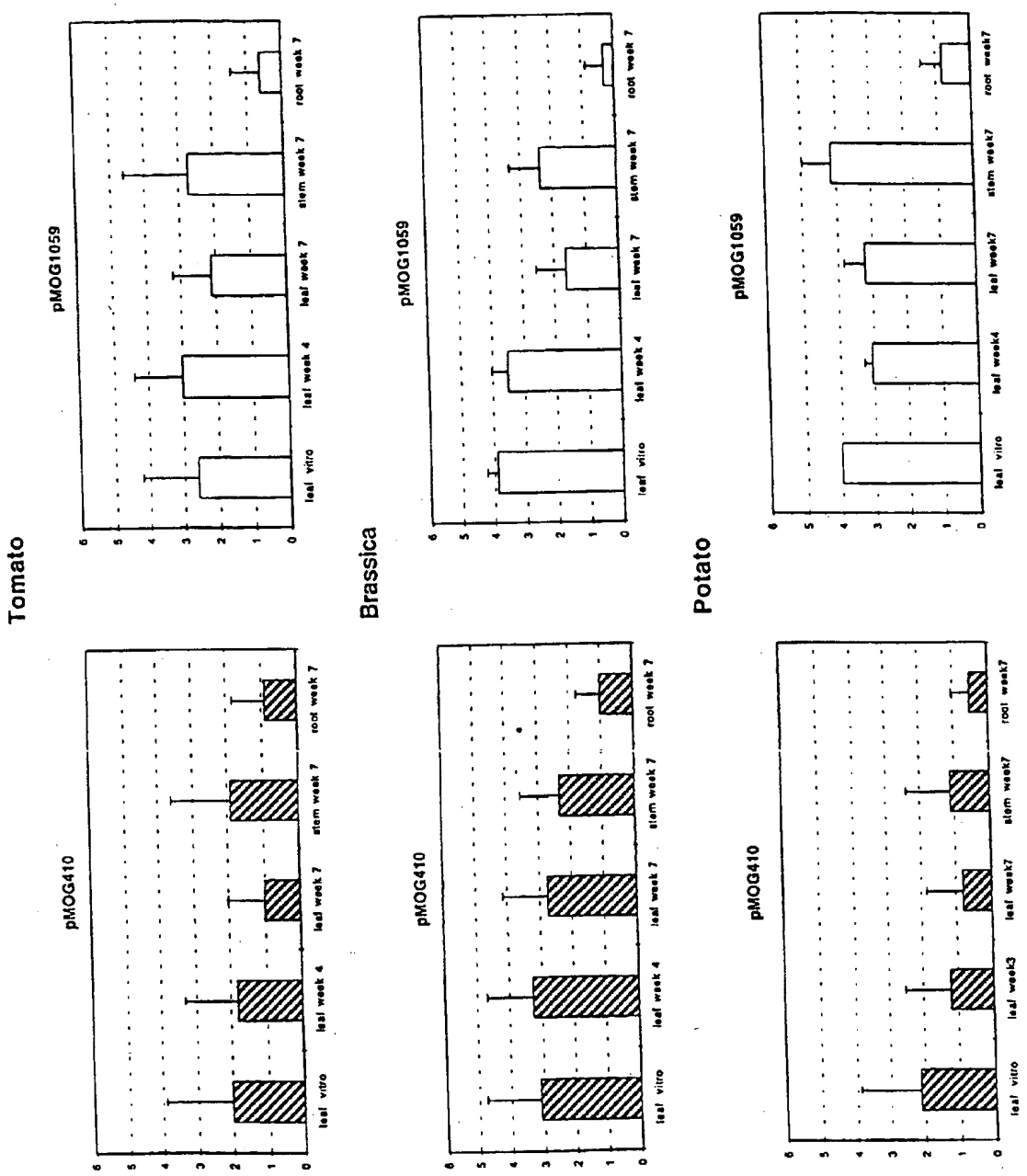
Tomato
Brassica
Potato
pMOG1059
pMOG410
leaf vitro
leaf week 4
leaf week 7
stem week 7
root week 7
leaf week3
leaf week4
leaf week7
stem week7
root week7

Fig. 3

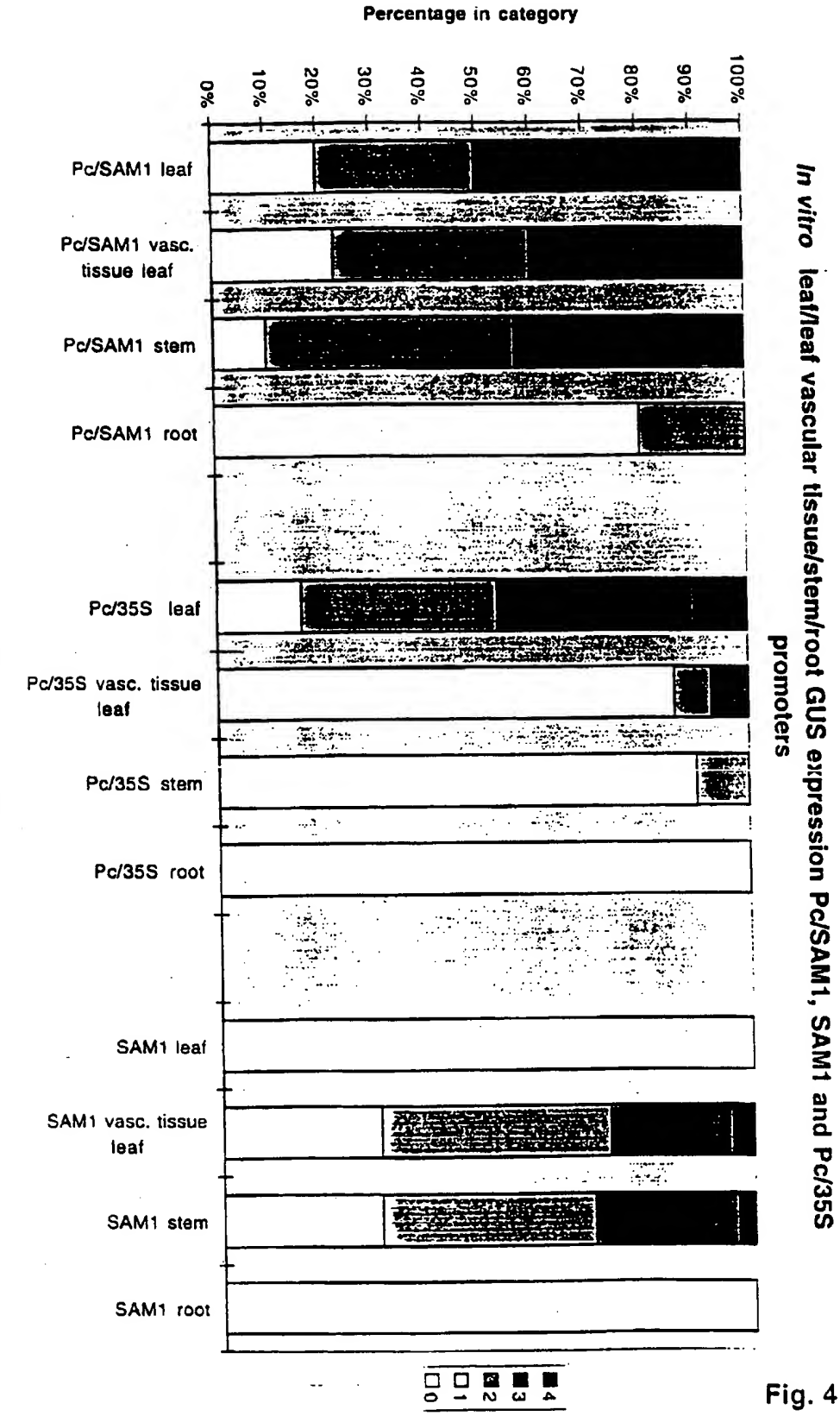
Percentage in category
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Pc/SAM1 leaf
Pc/SAM1 vasc. tissue leaf
Pc/SAM1 stem
Pc/SAM1 root
Pc/35S leaf
Pc/35S vasc. tissue leaf
Pc/35S stem
Pc/35S root
SAM1 leaf
SAM1 vasc. tissue leaf
SAM1 stem
SAM1 root
0 1 2 3 4
Construct and tissue
In vitro leaf/leaf vascular tissue/stem/root GUS expression Pc/SAM1, SAM1 and Pc/35S promoters
Page 1
Sheet1 Chart 3

Fig. 4

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Zeneca MOGEN
(B) STREET: Einsteinweg 97
(C) CITY: Leiden
(E) COUNTRY: The Netherlands
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(ii) TITLE OF INVENTION: New constitutive plant promoters

(iii) NUMBER OF SEQUENCES: 22

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 97203912.7
(B) FILING DATE: 12-DEC-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACTGAAGTG TGAAGGTGGA GATTATGTAT TCACTTGTTG ATTTGGTATA CATTCTATGT 60

AAGGTTCAAT TATTTACGTT ATATAATTAT AATGGAGTAA TTTACAGTAA TTGGGTTAAA 120

ATGGTTTGAT TCGGTCAGGT TGATACGGTT TGGAAGTTAA ACCCGGCCTA GATATGATGT 180

TACAACCAGT CCACATCTTT TATGATTTTA GTGGAACAAA CGAAGAGTTA TTTAGACGAT 240

ACAAACAAGG TCCGAATAAG TGTGAGCTGT CCCAAGTAAG ACCACGTAAT ACTCACCTCA 300

ACAAGATAGT GTTCTTAAAG TGTGTCAAAC ACAATCACAC ACACACAAAT CATAAAACAC 360

AAAGACGATA ATCCATCGAT CCACAGAATA GACGCCACGT GGTAGATAGG ATTCTCACTA 420

AAAAGTTCTC ACCTTTTAAT CTTTCTCCAC GCCATTTCCA CAAGCCATAA TCCTCAAAAA 480

TCTCAACTTT ATCTCCCAAA ACACAAATCT AGAAACCATG 520

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCCACTACAA TGAATTTGTT CGTGAACTAT TAGTTGCGGG CCTTGGCATC CGACTACCTC 60

TGCGGCAATA TTATATTCCC TGGGCCCACC GTGAACCCAA TTTCGCCTAT TTATTCATTA 120

CCCCCATTAA CATTGAAGTA GTCATGATGG GCCTGCAGCA CGTTGGTGAG GCTGGCACAA 180

CTCATCCATA TACTTTCTGA CCGGATCGGC ACATTATTGT AGAAAACGCG GACCCACAGC 240

GCACTTTCCA AAGCGGTGCC GCGTCAGAAT GCGCTGGCAG AAAAAAATTA ATCCAAAAGT 300

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 840 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATCCGAGC TTGCATGCCC CCACTACAAT GAATTTGTTC GTGAACTATT AGTTGCGGGC 60

CTTGGCATCC GACTACCTCT GCGGCAATAT TATATTCCCT GGGCCCACCG TGAACCCAAT 120

TTCGCCTATT TATTCATTAC CCCCATTAAC ATTGAAGTAG TCATGATGGG CCTGCAGCAC 180

GTTGGTGAGG CTGGCACAAC TCATCCATAT ACTTTCTGAC CGGATCGGCA CATTATTGTA 240

GAAAACGCGG ACCCACAGCG CACTTTCCAA AGCGGTGCCG CGTCAGAATG CGCTGGCAGA 300

AAAAAATTAA TCCAAAAGTG ACTGAAGTGT GAAGGTGGAG ATTATGTATT CACTTGTTGA 360

TTTGGTATAC ATTCTATGTA AGGTTCAATT ATTTACGTTA TATAATTATA ATGGAGTAAT 420

TTACAGTAAT TGGGTTAAAA TGGTTTGATT CGGTCAGGTT GATACGGTTT GGAAGTTAAA 480

CCCGGCCTAG ATATGATGTT ACAACCAGTC CACATCTTTT ATGATTTTAG TGGAACAAAC 540

GAAGAGTTAT TTAGACGATA CAAACAAGGT CCGAATAAGT GTGAGCTGTC CCAAGTAAGA 600

CCACGTAATA CTCACCTCAA CAAGATAGTG TTCTTAAAGT GTGTCAAACA CAATCACACA 660

CACACAAATC ATAAAACACA AAGACGATAA TCCATCGATC CACAGAATAG ACGCCACGTG 720

GTAGATAGGA TTCTCACTAA AAAGTTCTCA CCTTTTAATC TTTCTCCACG CCATTTCCAC 780

AAGCCATAAT CCTCAAAAAT CTCAACTTTA TCTCCCAAAA CACAAATCTA GAAACCATGG 840

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 477 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGATCCTGCA GCGATTTCAT TTTAGATTCT CAAAAATATT CTCAGATGTG TGGGATTTGA 60

GTAGAGTTTA TGTTGCGTTG GCATGATTTG AATAGTATGC AAGATTTTTG AGATTTTGCA 120

TTCGTTCATG TGTGTATGTG TGATTGTAGC TTGATATGAT TTAACCTGTT AGTTAAATGT 180

GCATAGACAA TAAGTAACAT ACGAAGCGAG TCACTAAGCA TAAGAGTCAA CTTGTTTTGC 240

TGAAAAGATA TCACTTATGA TTTTCGAATC ATTTTAGCTT TTTTGTCACT TGAGCTTAAT 300

GATTCTTCTG AAATTCGATT CTTTGTTTGG TTTATGTCAC ATTCTTTAGA ATTGAGAATC 360

TAAGAAATGC TTACAGGATA TGGTGAAACT ATTCTTTTAA GATAGCATGA TGCTTCTTTT 420

ATGATTCTAC AGTGGCTAAG TCATTTTTTT TTTGTTCTAT TCTTTGTAGC ACCATGG 477

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGATTTGTAT TGCAGCGATT TCATTTTAG 29

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATCTGGTCAC AGAGCTTGTC 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTCTCCATGG TGCTACAAAG AATAG 25

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGGGATCCTG CAGCGATTTC ATTTTAG 27

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACATGAACGA ATGCAAAATC TC 22

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGATTTTGCA TTCGTTCATG TG 22

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TGTAAGCATT TCTTAGATTC TC 22

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AAGAAATGCT TACAGGATAT GG 22

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GACAAGCTTG ATCCCATGGT GCTACAAAGA ATAG 34

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

AGTGGTACCA TCATAATACT CATCCTCCTT C 31

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CGAAGCTTTA CAAATCTAAT TTCATCACTA AATCGGA 37

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCTGCAATAC AAATCTAATT TCATCACTAA ATCGG 35

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 876 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATCATAATAC TCATCCTCCT TCTCAAGGTT CGTACGTATT ATCAATATCT AGTATATACT 60

TGTCTTTGTT CTATGCTTTA TATCATCATT TTATGACAAA AAATGATTAA GGTCTTAGTT 120

AATGATTATG TATATGTGAA ACTTATATTT AGGGGCACAA TTTAATTTCG TATGATAATT 180

GTCTAGTTAG CTTTATGTAC TTATCATAAA AACCTTAGTG TTTATCGCAA TACTTTTCAA 240

ATATAGTGTA GAATCATAAT GGTCCCACTG TCATTATGTT TGATGCAAAT CTATTTGGAT 300

TTTGTTGGAT AATAAACCGA TGACGTGGAC CAGACCAGTA GCTATAAGAT TTGGTTCACA 360

TAGAAATTTT TTATAAGATA ATGTATCTAG GTTTGCTTAT GATTATACAT GTGATATTTA 420

ATACATGGCA CAGGTTCGTC GAGTTTCACA GCCATAGGTA CAATAGAAGG CAAATTCGAT 480

TGTGGTTATC TGGTAAAAGT TAAGTTGGGC TCAGAGATTC TTAACGGCGT TCTTTATCAT 540

TCGGCCCAGC CCGGCCCATC ATCATCTCCA ACCGCTGTTC TAAACAATGC CGTTGTACCT 600

TATGTTGAAA CTGGGAGGAG ACGGCGTCGT TTAGGTAAAA GACGAAGAAG CAGACGCAGA 660

GAAGATCCGA ATTACCCGAA ACCGAACCGG AGCGGTTACA ATTTCTTCTT TGCTGAGAAA 720

CATTGCAAGC TCAAATCACT TTATCCCAAC AAGGAGAGAG AGTTTACGAA ACTTATCGGA 780

GAATCGTGGA GCAATCTCTC TACCGAAGAA CGAATGGTAA CAAATTATCT TTTAAACCGT 840

TACCGATTTA GTGATGAAAT TAGATTTGTA GTAAAT 876

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGATCCCCGG GTACCATCAT AATACTCATC CTCCTTCTCA AGGTTCGTAC GTATTATCAA 60

TATCTAGTAT ATACTTGTCT TTGTTCTATG CTTTATATCA TCATTTTATG ACAAAAAATG 120

ATTAAGGTCT TAGTTAATGA TTATGTATAT GTGAAACTTA TATTTAGGGG CACAGTTTAA 180

TTTCGTATGA TAATTGTCTA GTTAGCTTTA TGTACTTATC ATAAAAACCT TAGTGTTTAT 240

CGCAATACTT TTCAAATATA GTGTAGAATC ATAATGGTCC CACTGTCATT ATGTTTGATG 300

CAAATCTATT TGGATTTTGT TGGATAATAA ACCGATGACG TGGACCAGAC CAGTAGCTAT 360

AAGATTTGGT TCACATAGAA ATTTTTTATA AGATAATGTA TCTAGGTTTG CTTATGATTA 420

TACATGTGAT ATTTAATACA TGGCACAGGT TCGTCGAGTT TCACAGCCAT AGGTACAATA 480

GAAGGCAAAT TCGATTGTGG TTATCTGGTA AAAGTTAAGT TGGGCTCAGA GATTCTTAAC 540

GGCGTTCTTT ATCATTCGGC CCAGCCCGGC CCATCATCAT CTCCAACCGC TGTTCTAAAC 600

AATGCCGTTG TACCTTATGT TGAAACTGGG AGGAGACGGC GTCGTTTAGG TAAAAGACGA 660

AGAAGCAGAC GCAGAGAAGA TCCGAATTAC CCGAAACCGA ACCGGAGCGG TTACAATTTC 720

TTCTTTGCTG AGAAACATTG CAAGCTCAAA TCACTTTATC CCAACAAGGA GAGAGAGTTT 780

ACGAAACTTA TCGGAGAATC GTGGAGCAAT CTCTCTACCG AAGAACGAAT GGTAACAAAT 840

TATCTTTTAA ACCGTTACCG ATTTAGTGAT GAAATTAGAT TTGTATTGCA GCGATTTCAT 900

TTTAGATTCT CAAAAATATT CTCAGATGTG TGGGATTTGA GTAGAGTTTA TGTTGCGTTG 960

GCATGATTTG AATAGTATGC AAGATTTTTG AGATTTTGCA TTCGTTCATG TGTGTATGTG 1020

TGATTGTAGC TTGATATGAT TTAACCTGTT AGTTAAATGT GCATAGACAA TAAGTAACAT 1080

ACGAAGCGAG TCACTAAGCA TAAGAGTCAA CTTGTTTTGC TGAAAAGATA TCACTTATGA 1140

TTTTCGAATC ATTTTAGCTT TTTTGTCACT TGAGCTTAAT GATTCTTCTG AAATTCGATT 1200

CTTTGTTTGG TTTATGTCAC ATTCTTTAGA ATTGAGAATC TAAGAAATGC TTACAGGATA 1260

TGGTGAAACT ATTCTTTTAA GATAGCATGA TGCTTCTTTT ATGATTCTAC AGTGGCTAAG 1320

TCATTTTTTT TTTGTTCTAT TCTTTGTAGC ACCATGG 1357

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GTCTTGTACA AATCTAATTT CATCACTAAA TCGG 34

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TTAGATTTGT ACAAGACCCT TCCTCTATAT AAGG 34

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TTCCCAGTCA CGACGTTGT 19

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1006 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGATCCCCGG GTACCATCAT AATACTCATC CTCCTTCTCA AGGTTCGTAC GTATTATCAA 60

TATCTAGTAT ATACTTGTCT TTGTTCTATG CTTTATATCA TCATTTTATG ACAAAAAATG 120

ATTAAGGTCT TAGTTAATGA TTATGTATAT GTGAAACTTA TATTTAGGGG CACAGTTTAA 180

TTTCGTATGA TAATTGTCTA GTTAGCTTTA TGTACTTATC ATAAAAACCT TAGTGTTTAT 240

CGCAATACTT TTCAAATATA GTGTAGAATC ATAATGGTCC CACTGTCATT ATGTTTGATG 300

CAAATCTATT TGGATTTTGT TGGATAATAA ACCGATGACG TGGACCAGAC CAGTAGCTAT 360

AAGATTTGGT TCACATAGAA ATTTTTTATA AGATAATGTA TCTAGGTTTG CTTATGATTA 420

TACATGTGAT ATTTAATACA TGGCACAGGT TCGTCGAGTT TCACAGCCAT AGGTACAATA 480

GAAGGCAAAT TCGATTGTGG TTATCTGGTA AAAGTTAAGT TGGGCTCAGA GATTCTTAAC 540

GGCGTTCTTT ATCATTCGGC CCAGCCCGGC CCATCATCAT CTCCAACCGC TGTTCTAAAC 600

AATGCCGTTG TACCTTATGT TGAAACTGGG AGGAGACGGC GTCGTTTAGG TAAAAGACGA 660

AGAAGCAGAC GCAGAGAAGA TCCGAATTAC CCGAAACCGA ACCGGAGCGG TTACAATTTC 720

TTCTTTGCTG AGAAACATTG CAAGCTCAAA TCACTTTATC CCAACAAGGA GAGAGAGTTT 780

ACGAAACTTA TCGGAGAATC GTGGAGCAAT CTCTCTACCG AAGAACGAAT GGTAACAAAT 840

TATCTTTTAA ACCGTTACCG ATTTAGTGAT GAAATTAGAT TTGTACAAGA CCCTTCCTCT 900

ATATAAGGAA GTTCATTTCA TTTGGAGAGG ACACGTATTT TTACAACAAT TACCAACAAC 960

AACAAACAAC AAACAACATT ACAATTACTA TTTACAATTA CCATGG 1006

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Mogen International N.V.
Einsteinweg 97
2333 CB LEIDEN
Nederland

name and address of the party to whom the viability statement is issued

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Mogen International N.V. Address: Einsteinweg 97 2333 CB LEIDEN Nederland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: CBS 414.93 Date of the deposit or of the transfer: [1] Thursday, 12 August 1993

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on Friday, 13 August 1993 [2]. On that date, the said microorganism was

[X] [3] viable

[ ] [3] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY HAS BEEN PERFORMED [4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Centraalbureau voor Schimmelcultures Address: Oosterstraat 1 P.O. Box 273 3740 AG BAARN The Netherlands	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): drs F.M. van Asma Date: Friday, 13 August 1993

[4] Fill in if the information has been requested and if the results of the test were negative.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/EP 98/08162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 106 739 A (COMAI LUCA ET AL) 21 April 1992 see the whole document ---	1,2
X	WO 92 18625 A (MOGEN INT) 29 October 1992 * see esp. p.10 l.19-37 * ---	1,2
X	EP 0 559 603 A (SANDOZ AG ;SANDOZ LTD (CH); SANDOZ AG (DE)) 8 September 1993 * see esp. p.3 l.49-57 * ---	1,2
X	WO 95 14098 A (CUI DECAI ;BIOTECHNOLOGY RES & DEV (US); NI MIN (US); GELVIN STANT) 26 May 1995 * see esp. p.11 l.8 - p.16 l.4, p.22 l.12 - p.23 l.5 * --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 1999	08/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/EP 98/08162

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
L	KONONOWICZ H. ET AL.: "Subdomains of the octopine synthase upstream activating element direct cell-specific expression in transgenic tobacco plants" THE PLANT CELL, vol. 4, no. 1, January 1992, pages 17-27, XP002065097 * this document was cited to confirm the expression pattern conferred by the ocs UAS disclosed in the prior art document WO95/14098 *	1-3
A	US 5 097 025 A (BENFEY PHILIP N ET AL) 17 March 1992 see the whole document	1-10
A	WO 94 12015 A (CHUA NAM HAI) 9 June 1994 cited in the application * see esp. p.6 l.9-27, p.23 l.1-5 *	1-10
A	WO 97 20056 A (CAMBRIDGE ADVANCED TECH ;GRAY JOHN CLINTON (GB); SANDHU JAGDEEP SI) 5 June 1997 see the whole document	9-15

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/EP 98/08162

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5106739 A	21-04-1992	CA 1334175 A	31-01-1995
WO 9218625 A	29-10-1992	AU 663871 B	26-10-1995
		AU 1698992 A	17-11-1992
		BR 9205894 A	27-09-1994
		CA 2105592 A	17-10-1992
		CZ 9302145 A	13-07-1994
		EP 0513884 A	19-11-1992
		HU 65482 A	28-06-1994
		IL 101592 A	27-12-1998
		JP 6506595 T	28-07-1994
		SK 111693 A	02-02-1994
EP 0559603 A	08-09-1993	AU 661359 B	20-07-1995
		AU 3110493 A	15-07-1993
		CA 2086863 A	10-07-1993
		CN 1079779 A	22-12-1993
		CZ 9203923 A	16-03-1994
		HU 69949 A	28-09-1995
		JP 5276951 A	26-10-1993
		MX 9300059 A	01-07-1993
		SK 392392 A	09-08-1995
		US 5612472 A	18-03-1997
		ZA 9300145 A	08-07-1994
WO 9514098 A	26-05-1995	AU 687961 B	05-03-1998
		AU 1288595 A	06-06-1995
		BR 9408093 A	12-08-1997
		CN 1136825 A	27-11-1996
		EP 0729514 A	04-09-1996
		JP 9505205 T	27-05-1997
US 5097025 A	17-03-1992	NONE	
WO 9412015 A	09-06-1994	JP 8503853 T	30-04-1996
		US 5723751 A	03-03-1998
WO 9720056 A	05-06-1997	AU 7635696 A	19-06-1997
		CA 2236166 A	05-06-1997
		EP 0870043 A	14-10-1998

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
46055 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/08162	10/12/1998	12/12/1997
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant MOGEN INTERNATIONAL NV et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/07/1999	13.03.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bilang, J Telephone No. +49 89 2399 8707

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/08162

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20 as originally filed

Claims, No.:

1-15 as received on 22/12/1999 with letter of 15/12/1999

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-15
	No:	Claims	1,2
Inventive step (IS)	Yes:	Claims	3-15
	No:	Claims	1,2
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Additional remarks Item I

In the absence of the priority document and for the purpose of the present International Preliminary Examination Report the priority is assumed to be validly claimed.

Additional remarks Item IV

1. The international search has been drawn up in respect of the entire international application, but the IPEA finds that the application does not comply with the requirements of unity of invention (**Article 34(3)** and **Rules 13** and **68 PCT**).

2. An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.
Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same or corresponding special technical features, special technical features being such features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

   The technical relationship among the present subject-matter of claims 4-8 and 9-14 is "chimeric plant promoter produced by assembling parts of promoters". However, this relation cannot be considered to consist of a special technical feature as defined above because it is not novel. Chimeric plant promoters produced by assembling parts of promoters are known e.g. from WO95/14098 (see below).

   The contributions claimed in the present application which are allegedly made over the prior art are:
   1. Constitutive plant promoter produced by assembling parts of the ferredoxin and the rolD promoter (claims 4-8 and 1-3, 15 (partially));
   2. Constitutive plant promoter produced by assembling parts of the plastocyanin and the S-adenosyl-methionine promoter (claims 9-14 and 1-3, 15 (partially)).

   These contributions are not so linked as to form one single inventive concept. Hence, the present claims fall apart into at least two groups of inventions.

Additional remarks Item V

1. The present application discloses promoters which are functional in plants and which are assembled from parts of (known) promoters. In particular, different upstream activating sequences (UAS) having different tissue (or developmental) specificity are joined to a minimal promoter, resulting in a constitutive promoter.

2. The following documents were taken into consideration:

   D1 WO 95/14098
   D2 Kononowicz et al., The Plant Cell, 1992, vol. 4, p. 17-27
   D3 US 5,097,025

3. In view of the disclosures of D1 and D3, the IPEA considers that the subject-matter of claims 1 and 2 has been anticipated by the prior art and therefore contravene **Article 33(2)(3) PCT**.

3.1 Claim 2, which is dependent on claim 1, covers a promoter comprising a minimal promoter and transcription-activating elements (i.e. UAS) from a set of promoters which have complementary pattern and level of transcription, wherein the UAS do not exhibit an absolute tissue-specificity.

D1 discloses chimeric regulatory regions based on *Agrobacterium tumefactiens* opine synthase genes. Said regulatory regions comprise various UAS from opine synthase genes linked to a (minimal) promoter. In particular, the UAS are derived from the mannopine synthase promoter (mas) and from the octopine synthase promoter (ocs). It can be understood that the mas promoter directs weak expression in leaves, but strong expression in roots (p. 3, l. 20-21), whereas the ocs promoter directs strong expression in leaves (D2; referred to in D1). Chimeric regulatory regions comprising UAS from both the mas and ocs promoter (Figure 1) are constitutively expressed (Figs. 2 and 3).

Due to the unclear wording of the claims (see below), this authority maintains its opinion that the chimeric promoter disclosed in D1 comprising UAS from the mas and ocs promoters falls under the definition of claim 2.
As a consequence, claims 1 and 2 are not considered to fulfill the requirements of

Article 33(2)(3) PCT.

3.2 D3 discloses different synthetic promoters comprising various subdomains of the 35S promoter. Each subdomain confers a different tissue- and developmentally specific gene regulation (col. 3, l. 5-22). A combination of these constructs obviously leads to the (naturally occurring) 35S promoter.
The claims of the present application do not exclude the possibility that the "set of promoters" corresponds to a set of artificial promoters derived from one naturally occurring promoter. In its broadest sense D3 thus discloses a set of promoters, from which transactivating elements (the B-subdomains) can be chosen for combination. The transactivating elements have complementary pattern of transcription and give rise to different levels of expression. The 35S promoter, which comprises these transactivating elements and a minimal promoter thus is considered to fall under the definition of claim 1.

Additional remarks Item VIII

The expression "complementary pattern of transcription" is not clear (**Article 6, Rule 6 PCT**).
It would appear that two promoters may, for example, be complementary with respect to their pattern of expression at different developmental stages of a plant, but both promoters may drive expression in the same tissue of the mature plant. It thus may depend on the number of factors which are analysed whether two given promoters have "complementary pattern of transcription" or not.
Since the UAS do not exhibit absolute tissue-specificity, it is also unclear what differences in expression patterns two promoters should have in order to be considered "complementary". A promoter which is 100 times more active in leaves than in roots has different (complementary?) pattern of transcription than a promoter which is 500 times more active in leaves than in roots.

In addition, it seems that the expression "complementary level of transcription" does not make sense. It is questionable whether the skilled person would consider a weak promoter to have a "complementary level of expression" to a strong promoter.

CLAIMS

1. Plant promoter, characterized in that it comprises a minimal promoter and transcription-activating elements from a set of promoters, which elements have a complementary pattern and level of transcription in a plant.

2. Plant promoter according to claim 1, characterized in that each of the transcription-activating elements do not exhibit an absolute tissue-specificity, but mediate transcriptional activation in most plant parts at a level of ≥1% of the level reached in the part of the plant in which transcription is most active.

3. Plant promoter according to claim 1 or 2, characterized in that one promoter of the set of promoters is specifically active in green parts of the plant, while the other promoter is specifically active in underground parts of the plant.

4. Constitutive plant promoter according to claim 3, characterized in that it is a combination of the ferrodoxine and the RolD promoter.

5. Constitutive plant promoter of claim 4, characterized in that the minimal promoter element is derived from the ferredoxin promoter.

6. Constitutive plant promoter according to claim 4 or 5, characterized in that the ferredoxin promoter is derived from *Arabidopsis thaliana*.

7. Constitutive plant promoter according to claim 6, characterized in that it comprises the sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

8. Constitutive promoter according to claim 7, characterized in that it comprises the sequence of SEQ ID NO: 3.

9. Plant promoter according to claim 3, characterized in that it is a combination of the plastocyanin and the S-adenosyl-methionine-1 promoter.

10. Plant promoter according to claim 9, characterized in that the minimal promoter element is derived from the S-adenosyl-methionine-1 promoter.

11. Plant promoter according to claim 9 or 10, characterized in that the plastocyanin promoter is derived from *Arabidopsis thaliana*.

12. Plant promoter according to claim 9, 10 or 11, characterized in that the S-adenosyl-methionine-1 promoter is derived from *Arabidopsis thaliana*.

13. Plant promoter according to claim 12, characterized in that it comprises the sequences of SEQ ID NO:4 and SEQ ID NO:17.

14. Plant promoter according to claim 13, characterized in that it comprises the sequence of SEQ ID NO: 21.

15. Chimaeric gene construct for the expression of genes in plants comprising the promoter of any of claims 1-14.